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## Transitory Effects of Dimethyl Sulfoxide on the Electrical Properties of Isolated Frog Skin<sup>1</sup>

DAVID M. BAUMANN and JEWETT DUNHAM<sup>2</sup>

*Abstract.* The experiments indicate that when DMSO-Ringer's solutions are applied to the outside of the frog skin, the electrical potential difference across the skin decreases while the short circuiting current increases slightly. Ringer's solutions containing DMSO applied to the inside of the skin caused both the short circuiting current and the potential difference across the skin to decrease. These observations appear to be directly related to the concentration of DMSO-Ringer's applied. The observed effects are discussed with respect to possible changes in the skin brought about by the DMSO and the relationships of these phenomena to active transport functions of the skin.

In recent years there has been considerable interest and investigation of the biological effects produced by dimethyl sulfoxide, (DMSO). Experiments which explore the involvement of DMSO in biological carrier mechanisms and membrane penetration of certain chemical substances are reported by Block (1964) and by Klingman (1965). DMSO penetrates skin rapidly and has been topically applied to animals and humans as a curative substance for certain ailments. Inconsistent results in terms of success of the therapy have been reported. Due to detrimental effects, the use of this chemical on humans was terminated in 1965. The records of a symposium dealing with various aspects of DMSO are reported in the Annals of the New York Academy of Science of 1967.

One of the aspects of the effect of DMSO on biological barriers has been its effect on the electrical properties of isolated frog skin. Morain, Replogle and Curran (1966) have shown that DMSO applied to the outside of isolated frog skin causes the potential difference across the skin to decrease. The net short-circuiting currents determined over periods of exposure of about 60 minutes also showed a decrease. However, during the first 10 minutes there was an increase in the short-circuiting current, which was followed by the over-all decrease. The conductance across the skin showed an over-all increase, the change being most rapid during the first 10 minutes of exposure.

Franz and Van Bruggen (1967) found that the application of DMSO to the frog skin produced similar effects. The application of low concentrations (2.5%) of DMSO to the epidermal side re-

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sulted in decreases (25%-50%) of the electrical potential across the skin. They reported that the short-circuiting current usually showed a slight increases but that in some cases it showed a decrease or no change at all. Work on the relationship between osmotic changes and active sodium transport by Ussing and Windhager (1964) and Ussing (1965) indicates similar transient electrical results when the outside solution bathing isolated frog skin is made hypertonic with respect to the inner solution.

The experiments reported here involve the variation in electrical potential and the amount of short-circuiting current required to reduce the potential of the frog skin to zero when the skin was subjected for short periods of time to DMSO.

### METHODS

The ventral abdominal skin of a frog, *Rana pipiens*, was removed and placed between two symmetrical chambers. The chambers and associated connectors are similar to the apparatus used by Ussing and Zerahn (1951). The surface of the skin exposed to the solutions in the two chambers was 2.7 cm<sup>2</sup>. The amount of the bathing solution in each chamber was 25 ml. Contact with the solutions was made to calomel electrodes through 3 M KCl-agar bridges. The potential difference across the frog skin was recorded continuously with a potentiometric recorder. These millivolt potentiometric values of the skin were compared for accuracy with a calibrated oscilloscope. In some experiments the skin was soaked in Ringer's fluid for periods of 30 minutes to an hour before it was placed between the chambers. At intervals of about 2 minutes a short-circuiting current was applied through the solutions in each chamber, using Ag-AgCl electrodes. The amount of current required to reduce the skin potential to zero was determined. These values, determined with calibrated microammeter, were recorded on the continuous potentiometric record. Equilibration of the skin bathed by normal Ringer's solution on the apparatus was 30 minutes or longer. The equilibration time was dependent on the establishment of a relatively steady potential difference and the consistency of the short-circuiting current. Air was bubbled continuously through the apparatus to provide uniform oxygenation and mixing of the bathing fluids.

The Ringer's solution contained: NaHCO<sub>3</sub> 2.5 mM, NaCl 112 mM, KCl 2.0 mM, and CaCl 1.0 mM per liter of solution. The pH of this air-stirred solution was 8.2-8.3 and all experiments were run at room temperatures, 23° to 26°C. DMSO was added by pipette and was rapidly mixed by the air circulator. Ringer's and glucose solutions were substituted for DMSO in control experiments. The skins were subjected to DMSO concentrations of 0.0 (control), 0.05 M, 0.10 M, 0.15 M and 0.20 M. Control exper-

iments using 0.20 M glucose solutions were also performed. These concentrations were applied to the outside, the epidermal side or to the inside, the dermal surface of the frog skin.

After a short period of exposure of the skin to the experimental solutions, normally a ten minute period, the chambers were drained and rinsed three times with Ringer's solution. These washing periods were about 1 minute in duration. Subsequently the chambers were filled with normal Ringer's solution and the potential and short-circuiting current were recorded for about 15 minutes.

### RESULTS

The results reported here were obtained over a period of about a year. The data showed a wide variation in the apparent absolute values of the skin potentials and short-circuiting current measurements. The variability was so great that certain comparative aspects

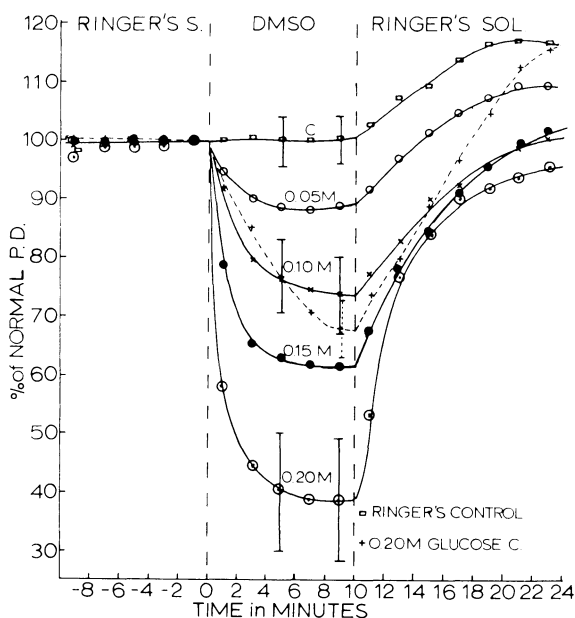


Fig. 1. Effect of DMSO on the potential difference, (P.D.), of isolated frog skin. DMSO was added to the outside Ringer's solution at time zero. Ringer's solution and glucose were used as controls. The points are average values. The final concentration of DMSO and the number of skins for each experiment was: 0.00M, (control, C), 10 skins; 0.05 M, 16 skins; 0.10 M, 17 skins; 0.15 M, 8 skins; 0.20 M, 11 skins. The dash-line curve is 0.20M glucose control (6 skins). DMSO-Ringer's and glucose-Ringer's solution was replaced by normal Ringer's solution after 10 minutes. Skins had been bathed in Ringer's solution prior to zero time. The bars on some points represent  $\pm 1$  S.E.M.

of the data cannot be considered statistically significant; therefore, the values only indicate trends.

The results are summarized on Figures 1, 2, 3, and 4. The curves are based upon the percent of change which occurred when the skin was subjected to the experimental solutions. The potential and short-circuiting current of the skin was considered to be at the 100 percent level at the point just prior to the addition of the test materials. Thus the ordinates at the beginning of each curve coincide, making it possible to compare the results. The values for the normal skin potential difference ranged from 12 to 86 millivolts and the short-circuiting currents ranged from 8 to 63 microamperes per centimeter squared. The points on the curve are the averages of the values determined. The number of skins for each of the concentrations is indicated in the legend for the figures. Standard errors of the means are indicated by vertical lines on the potential curves for a few of the points. The difference in the potentials between the Ringer's control, 0.10 M and 0.20 M DMSO concentrations appear to be significant (Fig. 1 and 2). The curves indicate that as the concentration of DMSO is increased, there is

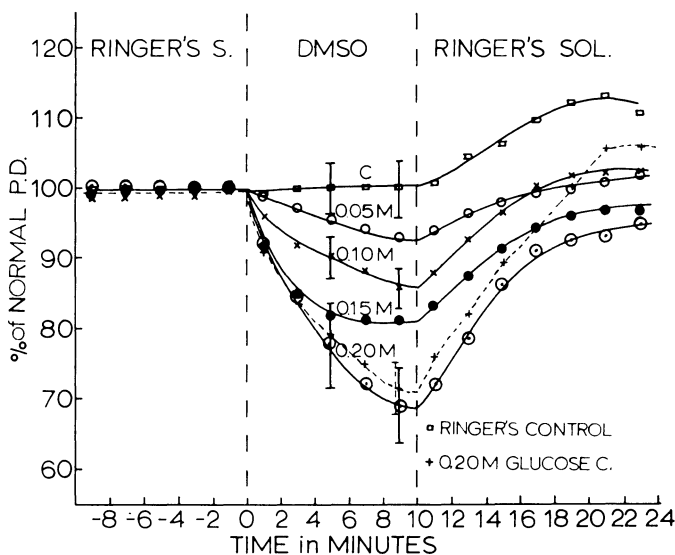


Fig. 2. Effect of DMSO on the potential difference, (P.D.), of isolated frog skin. DMSO was added to the inside Ringer's solution at time zero. Ringer's solution and glucose were used as controls. The points are average values. The final concentration of DMSO and the number of skins for each experiment was 0.00M, (control, C), 10 skins; 0.05 M, 10 skins; 0.10M, 8 skins; 0.15 M, 7 skins; 0.20 M, 11 skins. The dash-line curve is 0.20 M glucose control (7 skins). DMSO-Ringer's and glucose-Ringer's solution was replaced by normal Ringer's solution after 10 minutes. Skins had been bathed in Ringer's solution prior to zero time. The bars on some points represent  $\pm 1$  S.E.M.

a relative proportional decrease in the potential of the skin. The magnitude and the rate of change is different for the two sides of the skin. DMSO on the outside of the frog skin causes a rapid and more extensive drop in the potential when compared with its effect on the inside of the skin. The results of the 0.20 M glucose control are shown on figures 1 and 2.

The average values show an increase in the short-circuiting current when the outside of the skin is treated with DMSO. The glucose control shows a decrease in the short-circuiting current. (Fig. 3). In our DMSO experiments the current went up during this time interval in all cases except two, which showed no change. The results of treatment on the inside of the skin are relatively

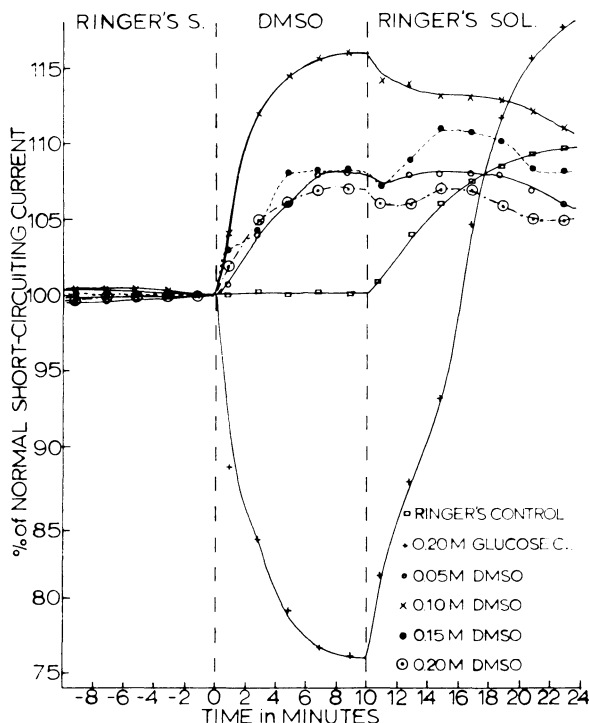


Fig. 3. Effect of DMSO on the short-circuiting current of isolated frog skin. DMSO was added to the outside Ringer's solution at time zero. Ringer's solution and glucose were used as controls. The points are average values. The final concentration of DMSO and the number of skins for each experiment was 0.00 M, (control, C), 10 skins; 0.05 M, 16 skins; 0.10 M, 17 skins; 0.15 M, 8 skins; 0.20 M, 11 skins. The glucose control curve is 0.20 M and represents the average of 6 skins. DMSO- Ringer's and glucose-Ringer's solution was replaced by normal Ringer's solution after 10 minutes. Skins had been bathed in Ringer's solution prior to zero time.

regular (Fig. 4), showing that the short-circuiting current decreases. The individual short-circuiting current curves are not statistically significant due to large variations in the values obtained. However, the short-circuiting effect is different, depending on the surface of application; either increasing or decreasing as shown by the short-circuiting current curves.

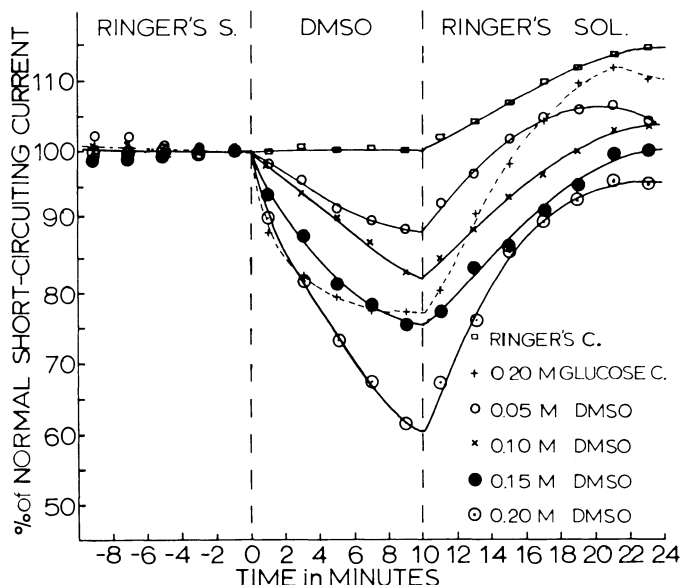


Fig. 4. Effect of DMSO on the short-circuiting current of isolated frog skin. DMSO was added to the inside Ringer's solution at time zero. Ringer's solution and glucose were used as controls. The points are average values. The final concentration of DMSO and the number of skins for each experiment was: 0.00 M, (control, C), 10 skins; 0.05M, 10 skins; 0.10 M, 8 skins; 0.15 M, 7 skins; 0.20 M, 11 skins. The dash-line curve is 0.20 M glucose control (7 skins). DMSO-Ringer's and glucose-Ringer's solution was replaced by normal Ringer's solution after 10 minutes. Skins had been bathed in Ringer's solution prior to zero time.

The recovery curves indicate a stimulatory effect due to washing-out the experimental solutions and reintroducing the normal Ringer's solution. The effect of this stimulation which usually caused the potential to go above the pretreatment level subsided over periods of an hour or longer to the original level. As a general rule, in those cases which were tested, skins which were subjected to the stronger concentrations often recovered to a lower level of potential and short-circuiting current when compared to the pretreatment values.

## DISCUSSION

The results of exposure of the epidermal surface of isolated frog skins to DMSO are similar to those reported by Morain, Replogle and Curran (1966) and in part to those of Franz and Van Bruggen (1967). Our experiments showed an increase in the short-circuiting current when the skin was subjected to DMSO on its outside surface with the exception of two cases where no change was observed.

The results indicate that the changes which occurred are different depending upon which surface of the skin was subjected to the DMSO. These differences are due, in part, to specific effects of the DMSO, but are partially effected by osmotic phenomena. The glucose control serves as an indication of the osmotic effect. The observed results are probably also affected by the rate of movement of the DMSO to the active sites where the changes in the electrical properties of the skin originate.

The ionic transport across the frog skin is affected by the application of DMSO. Permeability changes apparently affect the passive and/or active ion transport systems of the skin. The potential decreases when the skin is treated with the DMSO on the inside or on the outside. The relative effect on the potential difference across the skin is greater when this chemical is in contact with the outside of the skin. If the drop in potential were simply an osmotic effect then the difference between application on the outside and inside would be due primarily to morphological differences in this complex organ. It appears that passive changes in anion permeability, as indicated by Koefford-Johnsen, Levi and Using (1952) and Franz and Van Bruggen (1967), especially those of the chloride ion, are important in these potential alterations.

The rate of decrease of potential is more rapid with the DMSO on the outside. The difference in time sequence between the inside and outside application could be due to the specific DMSO effects on certain critical cells. However, it is more likely that the difference in the penetration rate of the DMSO to the determining cells in the skin is the primary factor in the potential difference rate variant. The DMSO may reach the critical layer of cells in the skin more rapidly from the outside because of their proximity to the outside layer. Assuming the effects observed are partly those of changes brought about by hypertonic solution, the time sequence is still in the proper order if the determining cells are near the outside layer. The fact that certain basal layers of the epithelium are responsible for the potential across the skin has been substantiated, as indicated by Hoshiko (1961). Simple dehydration of the cells resulting in their deformation could be one of the determining



factors in the potential changes. The particular location of these cells may be indicative of the rate change. The results show that the observed effects cannot be attributed exclusively to osmotic phenomena.

Some irreversible damage of cellular membranes or changes in normal junctions between the cells of the skin may be caused by the DMSO. The failure of the skin to completely recover its potential is an indication of such damage especially at higher concentrations of DMSO. Trial experiments using DMSO concentrations greater than 0.20 M resulted in a rapid drop in potential from which there was usually no recovery.

The mean, short-term effect of the DMSO on the outside of the isolated skin is to cause an increase in the short-circuiting current. As shown by Ussing and Zerahn (1951) this would indicate an increase in the net movement of the sodium-influx across the skin as a result of active sodium transport. Conversely, DMSO on the inside of the skin decreases the short-circuiting current, indicating a decrease in this active process. Morain, Replogle and Curran (1966) showed that after an increasing short-circuiting current phase of about 10 minutes with DMSO on the outside of the skin, there was a decrease in the current.

The transitory effect, stimulatory or inhibitory, of the DMSO on the active transport process of the skin is dependent upon the surface of application. It would appear that the physical location as well as certain functional aspects of the transporting mechanism are involved in the demonstrated phenomenon. It is possible that the DMSO causes some critical, physical changes in the mechanism of the active transporting systems of the skin.

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